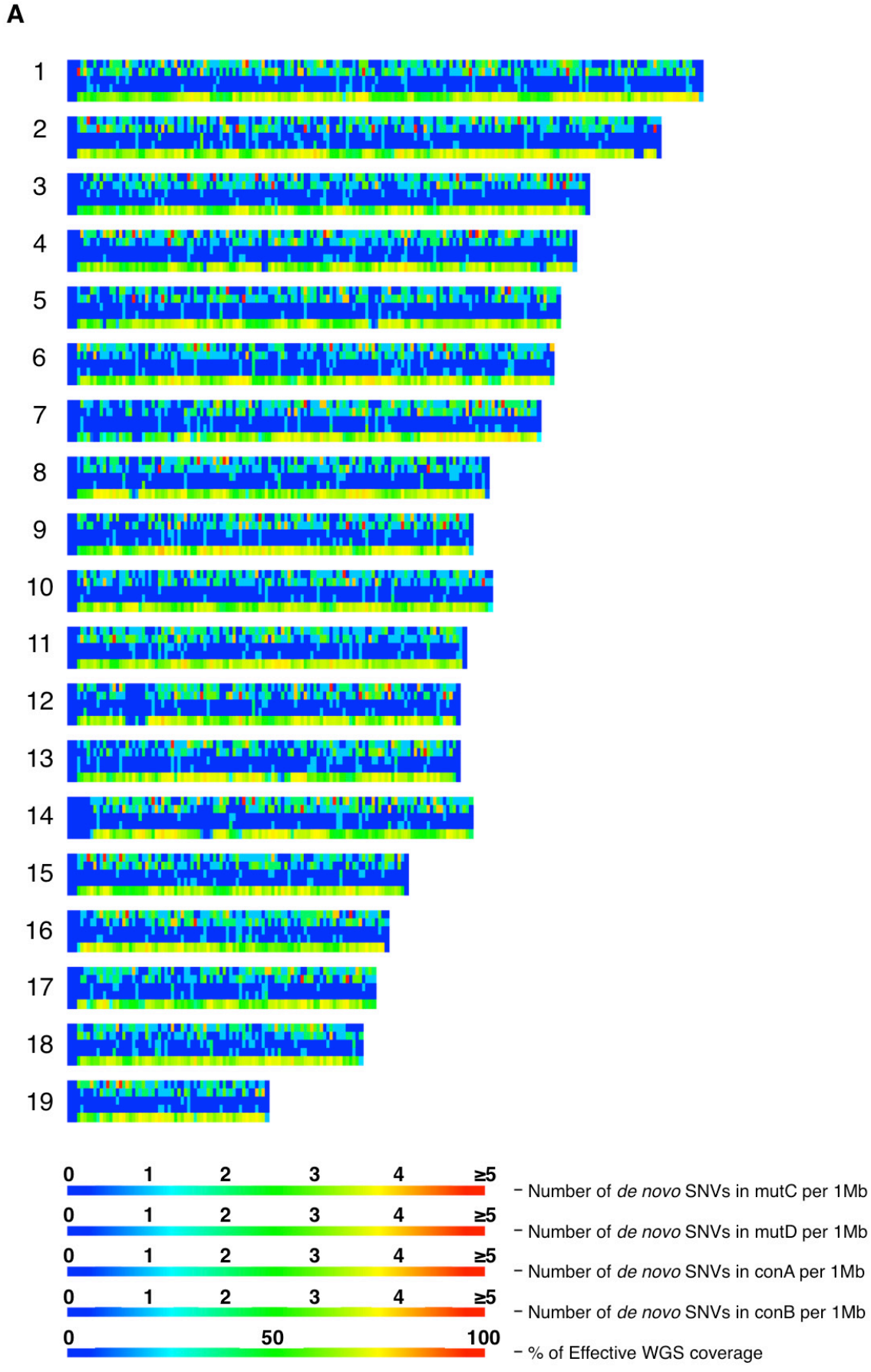
A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
0 1 2 3 4 ≥5
– Number of *de novo* SNVs in mutC per 1Mb
0 1 2 3 4 ≥5
– Number of *de novo* SNVs in mutD per 1Mb
0 1 2 3 4 ≥5
– Number of *de novo* SNVs in conA per 1Mb
0 1 2 3 4 ≥5
– Number of *de novo* SNVs in conB per 1Mb
0 50 100
– % of Effective WGS coverage

B

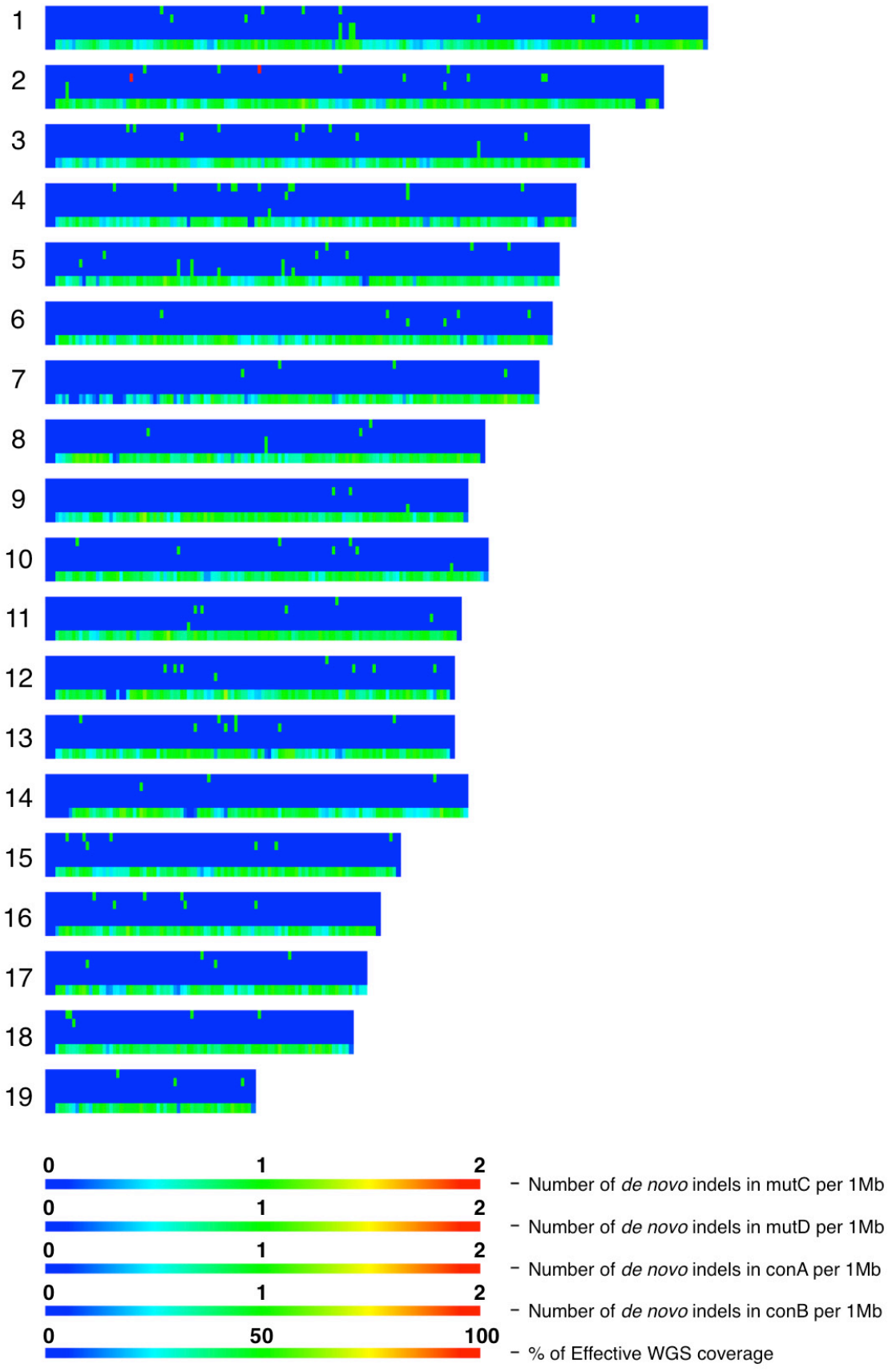
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
0
1
2
– Number of *de novo* indels in mutC per 1Mb
0
1
2
– Number of *de novo* indels in mutD per 1Mb
0
1
2
– Number of *de novo* indels in conA per 1Mb
0
1
2
– Number of *de novo* indels in conB per 1Mb
0
50
100
– % of Effective WGS coverage

Figure S1. *De novo* mutations observed in this breeding experiment. All *de novo* variants, both homozygous and heterozygous, on each of the autosomal mouse chromosomes are shown; SNVs are in **A**; indels are in **B**. Data shown for the control lines include all of the called homozygous and heterozygous variant candidates, including potential initial ancestral variants.

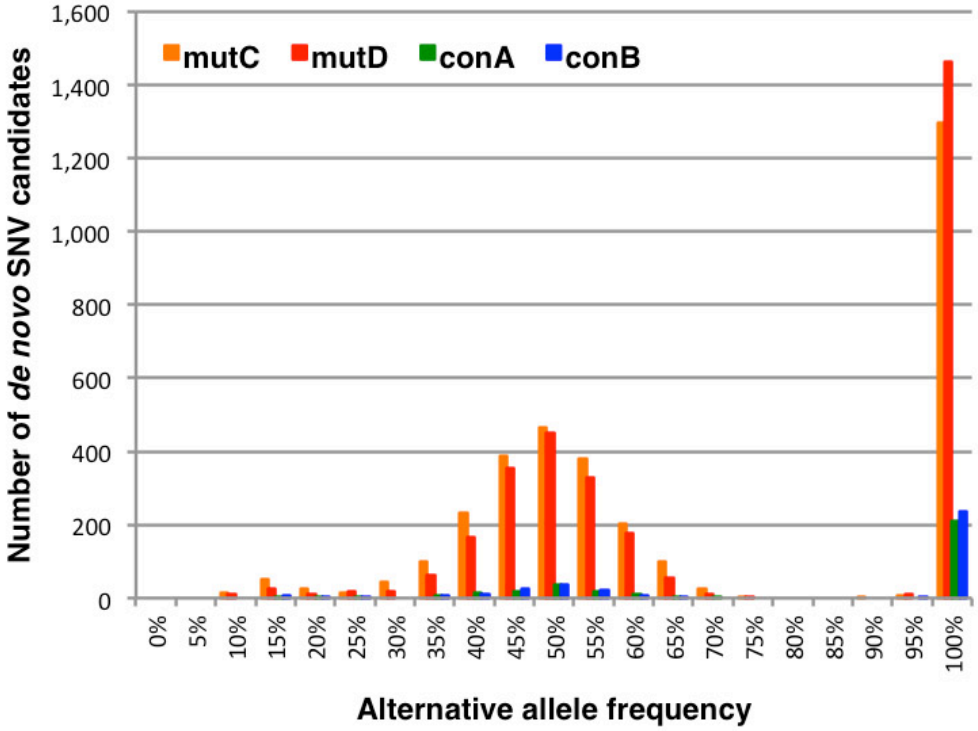

Figure S2. Distribution frequencies of called variants in the control and mutator breeding lines. Each bar indicates the number of called candidate *de novo* variants within a range of alternative allele frequencies. For example, the 50% bin represents the range: 47.5% $\leq x <$ 52.5%. We treated variants having the range: 25% $\leq x <$ 80% as heterozygotes and variants having the range: 80% $\leq x \leq$ 100% as homozygotes.

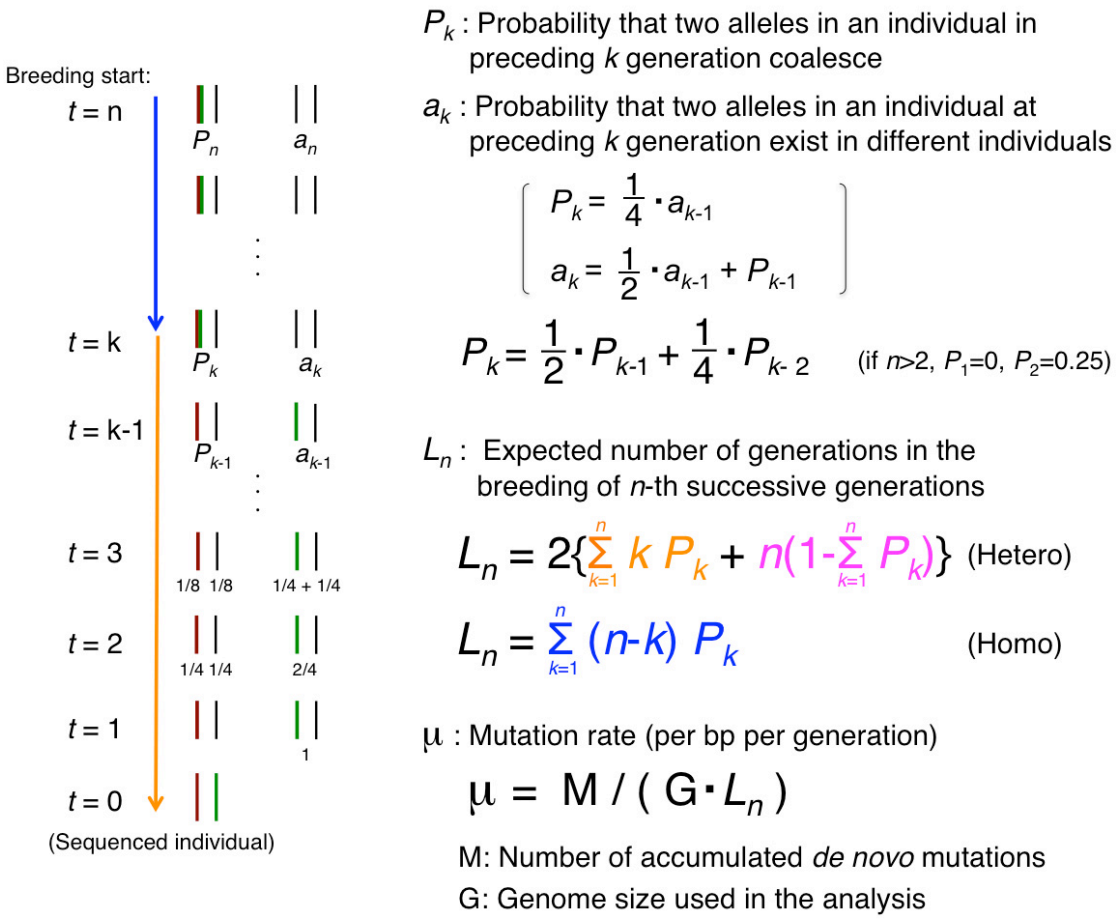

Figure S3. Method for estimating mutation rates. Estimation of per-generation mutation rates using the expected coalescence time of two alleles in a whole genome–sequenced individual: in the formula, colors represent the expected number of generations before (blue) and after (orange) coalescence, or when there is no coalescence during the breeding term (magenta). See Methods for more details.

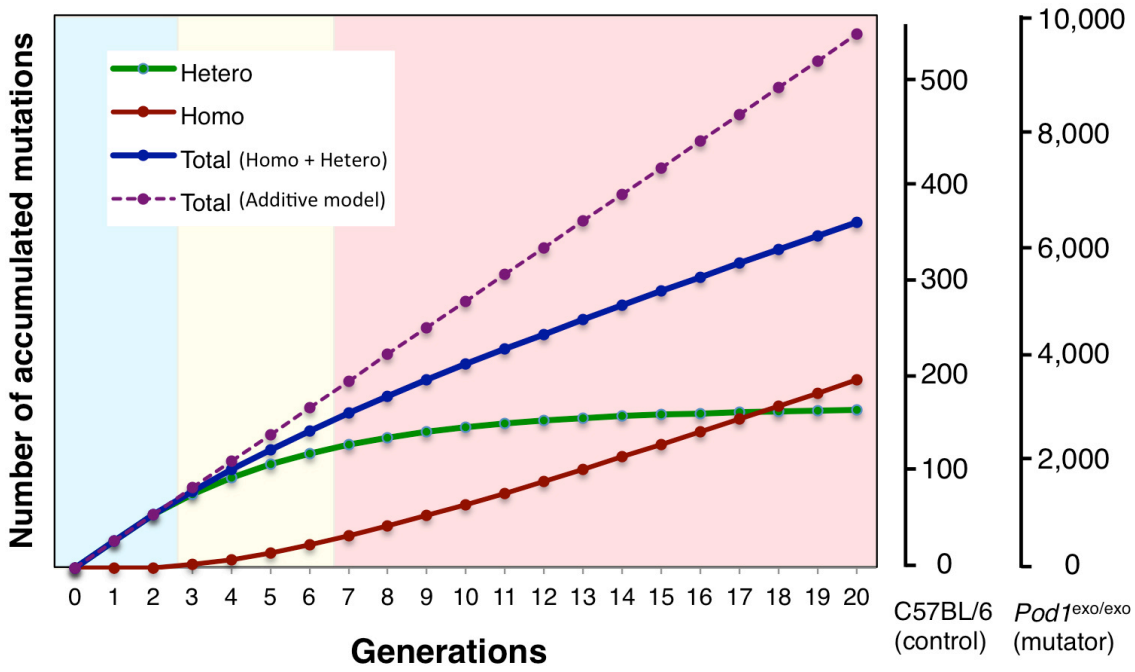

Figure S4. Process of *de novo* mutation accumulation. The number of *de novo* mutations predicted for each generation, based on estimated mutation rates, assuming that the mutation rate is constant during breeding and that all of the *de novo* mutations are inherited in a neutral fashion. The number of total accumulated mutations obeying the additive model (= 2×homozygous + 1×heterozygous) increases linearly through generations. Right axes: the number of mutations in control and mutator mice.

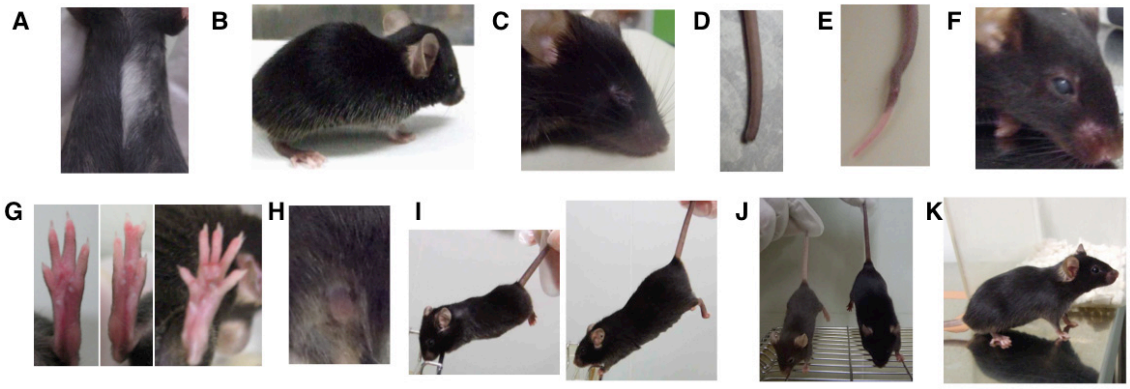

Figure S5. Phenotypic anomalies observed in mutator mouse lines. (A)-(F) Photographs of typical, frequently observed anomalies, including (A) minor color, (B) hydrocephaly, (C) closed eye, (D) cut tail, (E) tail kink, (F) cataract. (G)-(K) Inherited anomalies, including (G) syndactyly (right photograph shows a normal paw), (H) priapism, (I), short limbs and tail (right photograph shows a normal mouse), (J) color dilution (left), and (K) a human-audible vocalizer (Movie S1).

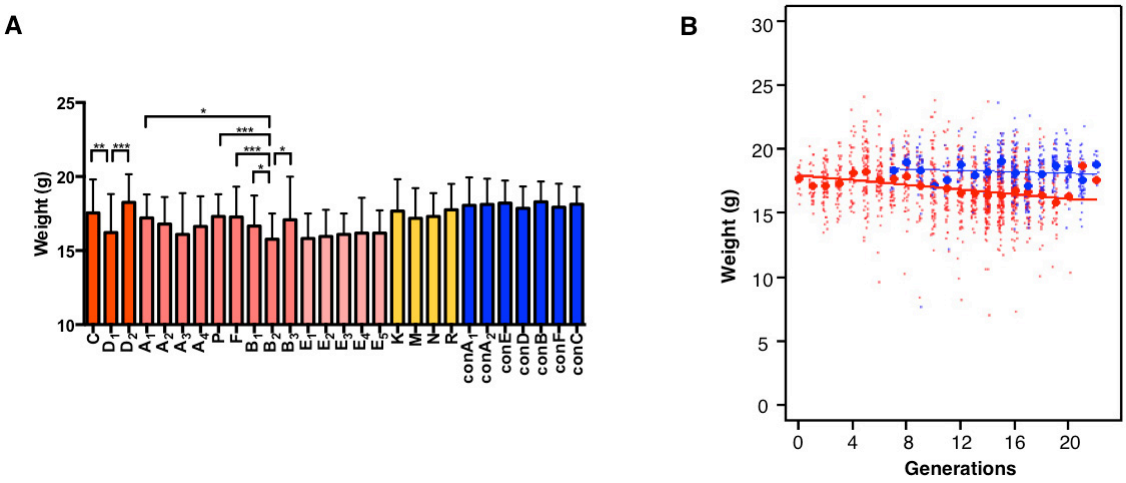

Figure S6. Body weights of 8-week-old female mice in the breeding lines. (A) Bar chart showing the body weights of female mice in control (blue) and mutator (red and yellow) breeding lines. Error bars represent standard deviations. Each data point represents the mean weight of individual mice from the grey-shaded generations in Fig. 1. Breeding-line names correspond to the sub-lines shown in Fig. 1. Asterisks, statistically significant differences between sub-lines belonging to the same subgroup ([C~D_2], [A_1~B_3], [E_1~E_5], [K~R] and [conA_1~conC]) determined by Tukey's multiple comparisons test ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). **(B)** Relationship between 8-week-old female body weight and generation number (red: mutator; blue: control). Solid line: simple linear regression of the posterior means.

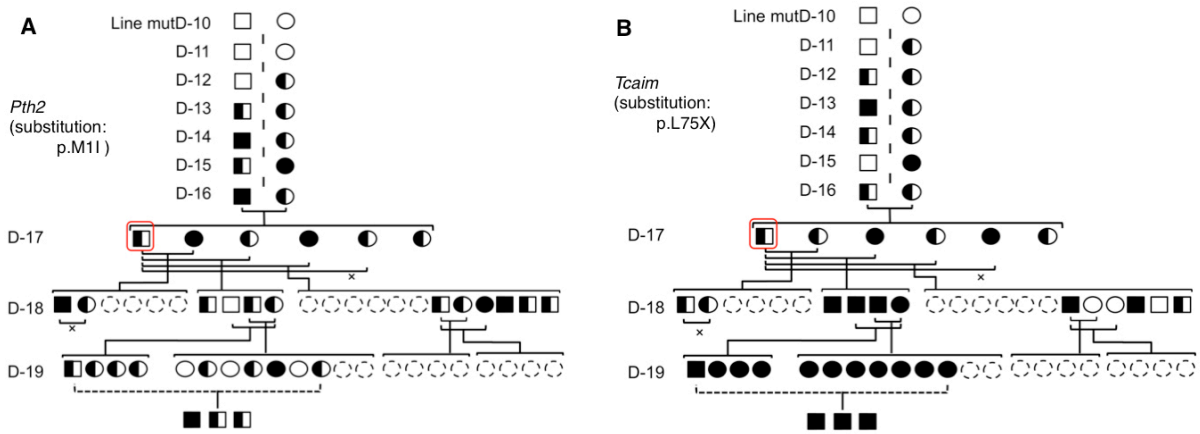

Figure S7. Pedigrees showing the genotyping results of two distinct *de novo* mutations. (**A**) A loss of start-codon mutation in the parathyroid hormone 2 (*Pth2*) gene and (**B**) a premature stop-codon mutation in the T cell activation inhibitor mitochondrial (*Tcaim*) gene, in the mutD line. Squares: males; circles: females. Filled: homozygotes; half-filled, heterozygotes; empty: no mutation detected. Dashed-line circles: postnatal deaths. ×: failure to reproduce. Dashed lines: non-sibling matings, which are shown here for reference. The effects of these two mutations were less serious than those of the *Itga8* mutation, shown in Fig. 4B.

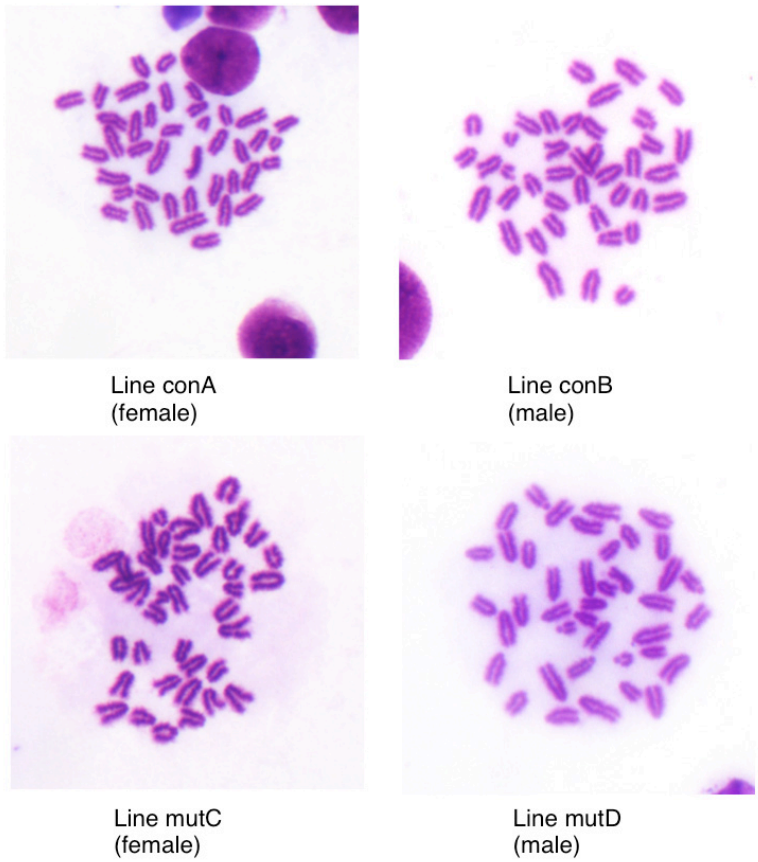

Figure S8. Representative karyotype analysis of control and mutator mice after long-term breeding. No chromosomal aberrations were detected in the control or mutator mice.

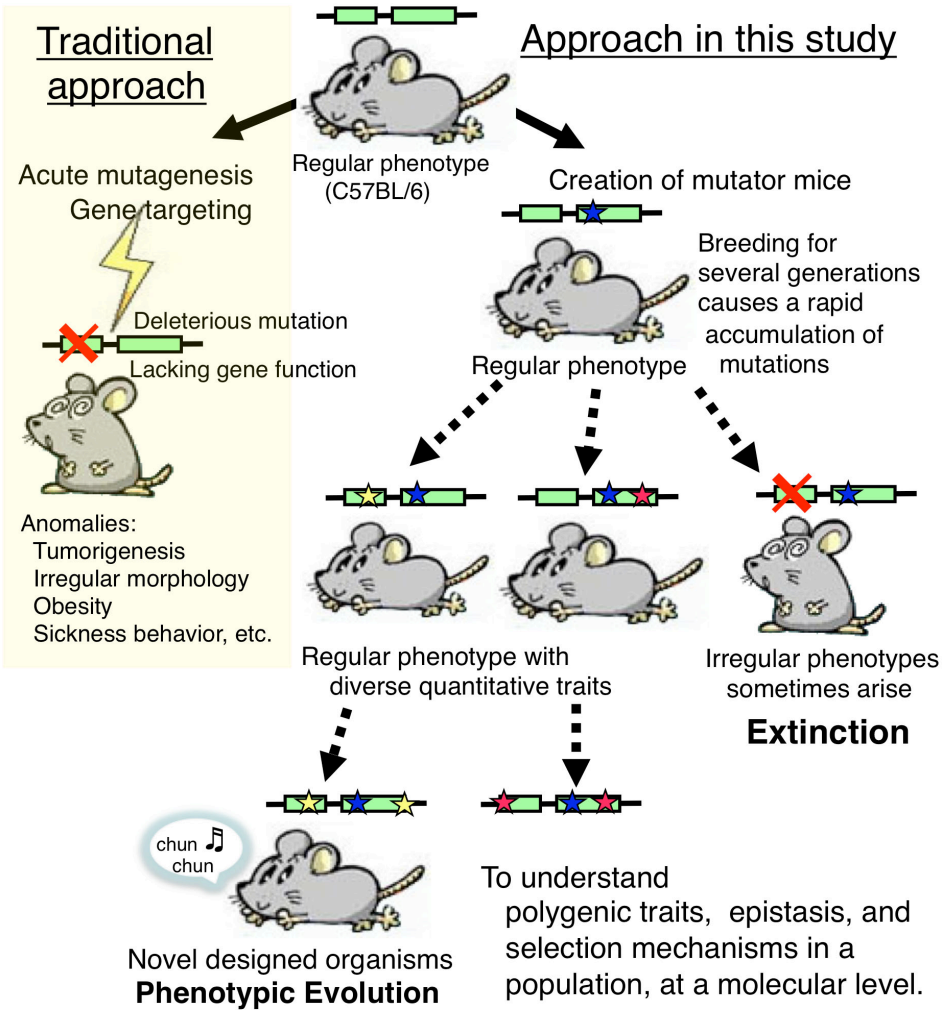

Figure S9. Strategy of the present study. The breeding of mutator mice that have an increased spontaneous germline mutation rate provides an efficient experimental model with which to study the expression of genetic variation and its maintenance in a population. Illustrations of mice were kindly provided by Dr. Masuya (RIKEN BRC, Japan).

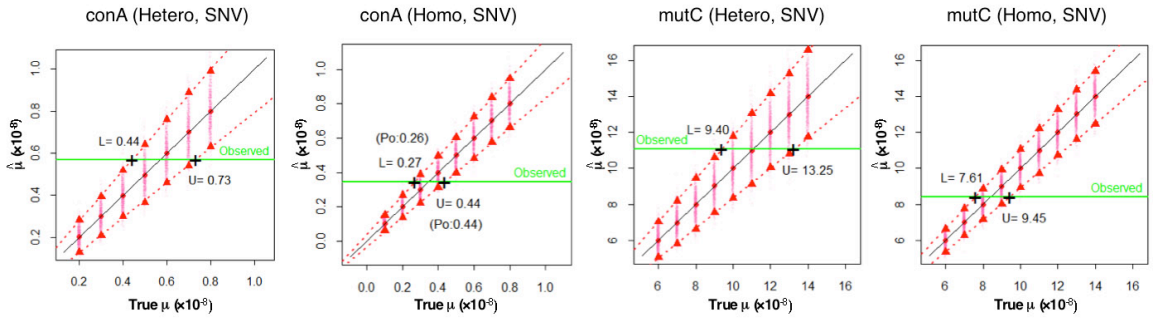

Figure S10. Examples of CIs for μ calculated by computer simulation. For details, see the Supplemental Methods, "Confidence Intervals for $\boldsymbol{\mu}$ and combined estimates." Green line: observed $\hat{\boldsymbol{\mu}}$; solid diagonal lines show $\hat{\boldsymbol{\mu}} = \boldsymbol{\mu}$; red circles: individual simulated values; red triangles: 2.5th and 97.5th percentiles of simulated values; red dashed lines: regression lines for the 2.5th and 97.5th percentiles; L (U): lower (upper) confidence limit for μ. Note that the simulated CI (0.27-0.44, $\times 10^{-8}$) for homozygous mutations in conA was essentially the same as that calculated by Poisson assumption (0.26-0.44, $\times 10^{-8}$).

Table S1.

Summary of sequencing conditions.

1. Reference

Species	Genome size (bp)	Except N (bp)	Build
mus musclus	2,730,871,774	2,652,783,500	UCSC mm10

2. Sequencing conditions, and the genome region covered by more than 5 effective reads

Sample	Read number	Total read (bp)	Read coverage (×)	More than 5× region (bp)	(%)
Adam	1,822,178,696	182,217,869,600	66.6	2,631,369,269	99.2%
Eve	2,956,330,210	347,357,853,267	127.1	2,553,495,843	96.3%
mutC	1,173,674,104	117,367,410,400	42.8	2,647,192,875	99.8%
mutD	1,566,489,730	156,648,973,000	57.5	2,645,093,070	99.7%
mutE	1,840,752,934	327,064,195,975	120.1	2,649,566,163	99.9%
conA	1,263,486,550	189,522,982,500	69.4	2,648,773,170	99.8%
conB	1,336,018,876	200,402,831,400	73.4	2,649,113,577	99.9%

3. Features of the EWC regions

Region	Total (bp)	CDS	UTR	Intron	Intergenic	ncRNA
Whole Genome (Autosome)	2,462,745,373	32,676,734	25,285,788	877,185,423	1,527,597,428	4,628,706
EWC (SNVs)	1,516,416,340	23,381,609	17,745,919	610,904,491	864,384,321	3,227,730
	61.6%	71.6%	70.2%	69.6%	56.6%	69.7%
EWC (indels)	961,909,845	23,009,088	15,727,883	419,997,153	503,175,721	2,562,087
	39.1%	70.4%	62.2%	47.9%	32.9%	55.4%

Table S2.

The number of eliminated variants from candidate *de novo* variants by using the sequencing results of "Adam/Eve" samples (see Methods). This filtration was useful to obtain credible candidate *de novo* mutations.

Sample	SNVs/ Indels	Homo/Hetero	Total called variants	*De novo* variants	Filtered variants
mutC	SNVs	Homo	3,009	1,304	1,705
		Hetero	2,399	1,944	455
	Indels	Homo	758	28	730
		Hetero	42	28	14
mutD	SNVs	Homo	3,246	1,472	1,774
		Hetero	2,058	1,633	425
	Indels	Homo	758	21	737
		Hetero	48	37	11
conA	SNVs	Homo	1,290	211	1,079
		Hetero	485	105	380
	Indels	Homo	721	10	711
		Hetero	14	5	9
conB	SNVs	Homo	1,320	235	1,085
		Hetero	494	103	391
	Indels	Homo	722	12	710
		Hetero	12	3	9

Table S3.

Results of validation by Sanger sequencing, and estimated mutation rates. SNV mutation rates were estimated by two different approaches: using the coalescent time and the number of accumulated mutations or using the number of mutations occurring in the final generation. Failures in the validation process were due to difficulties in primer design or Sanger sequencing conditions. The details for the PCR and Sanger sequencing analyses are shown in the Supplemental Material (Excel file). *Note that the homozygous variant numbers in the control lines were uncertain due to the small number of randomly tested variants used to discriminate between *de novo* and initial variants. NGS: next-generation sequencing.

			No. candidate variants	No. used for validation	Failed validation process	Initial variants	False in NGS result	Validated *de novo* variants	Occurred in the final generation	Expected *de novo* mutation number	Mutation rate ($\times 10^{-9}$)	Expected *de novo* number in the final generation	Mutation rate in the final generation ($\times 10^{-9}$)
SNV	**mutC**	Homo	1,304	10	0	-	0	10	-	1,304	84.3	-	-
		Hetero	1,944	30	0	-	0	30	7	1,944	110.6	453.6	150
	mutD	Homo	1,472	10	0	-	0	10	-	1,472	86.9	-	-
		Hetero	1,633	30	0	-	0	30	5	1,633	92.3	272.2	90
	conA	Homo	211	13	3	7	0	3	-	63.3*	3.4*	-	-
		Hetero	105	31	1	1	0	29	6	101.5	5.7	21.0	6.9
	conB	Homo	235	10	0	5	0	5	-	117.5*	5.1*	-	-
		Hetero	103	31	1	3	0	27	6	92.7	5.2	20.6	6.8
Indel	**mutC**	Homo	28	3	0	-	0	3	-	28	2.9	-	-
		Hetero	28	3	0	-	0	3	0	28	2.5	-	-
	mutD	Homo	21	3	0	-	0	3	-	21	2.0	-	-
		Hetero	37	3	0	-	0	3	0	37	3.3	-	-
	conA	Homo	10	3	0	1	0	2	-	6.7*	0.57*	-	-
		Hetero	5	5	0	0	1	4	1	4	0.35	-	-
	conB	Homo	12	3	0	2	0	1	-	4*	0.28*	-	-
		Hetero	3	3	0	0	0	3	1	3	0.26	-	-

Table S4.

Time spans of generations in four whole-genome-sequenced breeding lines. The long time spans of the 14th and 15th generations in mutC compared to the others might have increased the mutation rates estimated for this mouse line by using the number of heterozygous *de novo* mutations (including the final generation approach).

Generation number	Days / Generation			
	Line mutC	Line mutD	Line conA	Line conB
-1	81	81		
0	87	87	91	91
1	84	84	77	77
2	80	80	89	89
3	86	87	92	92
4	90	89	126	126
5	145	216	144	94
6	74	83	108	168
7	80	221	103	96
8	109	90	86	94
9	178	92	81	85
10	84	89	184	178
11	91	163	85	92
12	86	156	88	116
13	86	80	87	90
14	205	120	125	86
15	264	183	84	98
16	Sequencing	83	86	83
17		Sequencing	110	92
18			101	84
19			Sequencing	83
20				87
21				105
22				Sequencing
Averaged span	112.3	115.8	102.5	100.3

Table S5.

The spectrum of *de novo* SNVs. Mutation rates are per nucleotide per generation. Homozygous variants in the control lines were excluded because it was not possible to avoid contamination from the many initial variants. For the heterozygous variants in the control lines, only variants that were present in the original ancestral mouse lines of the randomly selected validated variants were excluded; ~70% of the variants listed in this table were not validated. This means that ~4.7% of the listed mutations include residual initial variants from the ancestral lines, which presumably had similar spectral features to the *de novo* ones.

				mutC				**mutD**				**conA**		**conB**	
	Ref	Alt		**Homo**		**Hetero**		**Homo**		**Hetero**		**Hetero**		**Hetero**	
				Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$
Total				**1,304**	**8.43**	**1,944**	**11.06**	**1,472**	**8.69**	**1,633**	**9.23**	**104**	**0.58**	**100**	**0.56**
Transition		**Total**		**565**	**3.65**	**767**	**4.37**	**642**	**3.79**	**641**	**3.62**	**68**	**0.38**	**71**	**0.39**
	G:C	A:T	non CpG	79	1.25	124	1.73	88	1.28	92	1.28	36	0.50	34	0.46
			CpG	27	10.56	43	14.81	32	11.43	42	14.36	18	6.12	20	6.73
	A:T	G:C	non CpG	459	5.15	600	5.93	522	5.35	507	4.97	14	0.14	17	0.16
Transversion		**Total**		**739**	**4.77**	**1,177**	**6.70**	**830**	**4.90**	**992**	**5.61**	**36**	**0.20**	**29**	**0.16**
	G:C	C:G	non CpG	18	0.29	26	0.36	20	0.29	29	0.40	4	0.06	5	0.07
			CpG	7	2.74	4	1.38	4	1.43	7	2.39	2	0.68	2	0.67
	G:C	T:A	non CpG	330	5.24	554	7.74	376	5.45	452	6.27	10	0.14	6	0.08
			CpG	11	4.30	16	5.51	8	2.86	14	4.79	0	0.00	3	1.01
	A:T	C:G	non CpG	276	3.09	447	4.41	301	3.08	379	3.72	7	0.07	7	0.07
	A:T	T:A	non CpG	97	1.09	130	1.28	121	1.24	111	1.09	13	0.13	6	0.06
GC to AT bias	G or C	A or T	(s→w)	447	6.82	737	9.90	504	7.02	600	8.00	64	0.85	63	0.83
	A or T	G or C	(w→s)	735	8.24	1,047	10.34	823	8.43	886	8.69	21	0.20	24	0.23
	(ratio: (s→w)/(w→s))				(0.83)		(0.96)		(0.83)		(0.92)		(4.14)		(3.57)

Table S6.

The spectrum of *de novo* indels. The number of indel mutations is shown in each column. "A:T" ("G:C") represents a single A:T (G:C) base pair insertion or deletion. ">2bp" represents the insertion or deletion of more than 2 base pairs. Parentheses show the number of variants that occurred on a repeat site, which is defined as a site containing multiple (>2) repetitive sequence elements. Homozygous variants in control lines were excluded as in Supplemental Table S5. All of the listed heterozygous variants in the control lines were confirmed by Sanger sequencing.

	mutC				**mutD**				**conA**		**conB**	
	Homo		**Hetero**		**Homo**		**Hetero**		**Hetero**		**Hetero**	
Total number	28		28		21		37		4		3	
	Insertion	Deletion	Insertion	Deletion	Insertion	Deletion	Insertion	Deletion	Insertion	Deletion	Insertion	Deletion
Total (on repeat site*)	21 (19)	7 (6)	19 (18)	9 (8)	19 (18)	2 (2)	23 (22)	14 (10)	0 (0)	4 (3)	1 (1)	2 (1)
A:T	19 (17)	3 (3)	17 (17)	3 (3)	15 (14)	1 (1)	22 (21)	8 (7)	0	1 (1)	1 (1)	1 (1)
G:C	2 (2)	3 (2)	1 (1)	6 (5)	3 (3)	1 (1)	1 (1)	3 (2)	0	2 (1)	0	0
>2bp	0	1 (1)	1 (0)	0	1 (1)	0	0	3 (1)	0	1 (1)	0	1 (0)

Table S7.

Details of the visible phenotypic anomalies observed in the breeding lines. The mutator breeding lines were also divided as follows: Start (generations 0-2), Early (generations 3-6), and Late groups (generations 7-22). Table **B** shows the details of the "other" phenotypes shown in Table **A**.

A

		n	Total abnormalities	Hydrocephalus	Minor color (white)	Cut tail	Tail kink	Closed eye	Cataract	Other
Wild-type										
	All	1,649	45 (2.7%)	8 (0.5%)	7 (0.4%)	10 (0.6%)	2 (0.1%)	3 (0.2%)	6 (0.4%)	9 (0.5%)
#0-22 (avg.13.4)	♂	867	18 (2.1%)	4 (0.5%)	6 (0.7%)	3 (0.3%)	1 (0.1%)	0 (0.0%)	1 (0.1%)	3 (0.3%)
	♀	782	27 (3.5%)	4 (0.5%)	1 (0.1%)	7 (0.9%)	1 (0.1%)	3 (0.4%)	5 (0.6%)	6 (0.8%)
Pold1$^{exo/exo}$										
	All	6,229	683 (11.0%)	123 (2.0%)	174 (2.8%)	69 (1.1%)	33 (0.5%)	67 (1.1%)	49 (0.8%)	168 (2.7%)
#0-22 (avg.10.5)	♂	3,138	347 (11.1%)	68 (2.2%)	109 (3.5%)	25 (0.8%)	15 (0.5%)	16 (0.5%)	20 (0.6%)	94 (3.0%)
	♀	3,091	336 (10.9%)	55 (1.8%)	65 (2.1%)	44 (1.4%)	18 (0.6%)	51 (1.6%)	29 (0.9%)	74 (2.4%)
Start group	All	709	29 (4.1%)	4 (0.6%)	3 (0.4%)	4 (0.6%)	3 (0.4%)	8 (1.1%)	2 (0.3%)	5 (0.7%)
#0-2 (avg.1.2)	♂	367	14 (3.8%)	3 (0.8%)	2 (0.5%)	1 (0.3%)	1 (0.3%)	2 (0.5%)	1 (0.3%)	4 (1.1%)
	♀	342	15 (4.4%)	1 (0.3%)	1 (0.3%)	3 (0.9%)	2 (0.6%)	6 (1.8%)	1 (0.3%)	1 (0.3%)
Early group	All	1,037	66 (6.4%)	9 (0.9%)	9 (0.9%)	6 (0.6%)	2 (0.2%)	6 (0.6%)	6 (0.6%)	28 (2.7%)
#3-6 (avg.4.6)	♂	559	30 (5.4%)	5 (0.9%)	6 (1.1%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	3 (0.5%)	14 (2.5%)
	♀	478	36 (7.5%)	4 (0.8%)	3 (0.6%)	6 (1.3%)	1 (0.2%)	5 (1.0%)	3 (0.6%)	14 (2.9%)
Late group	All	4,483	588 (13.1%)	110 (2.5%)	162 (3.6%)	59 (1.3%)	28 (0.6%)	53 (1.2%)	41 (0.9%)	135 (3.0%)
#7-22 (avg.13.3)	♂	2,212	303 (13.7%)	60 (2.7%)	101 (4.6%)	24 (1.1%)	13 (0.6%)	13 (0.6%)	16 (0.7%)	76 (3.4%)
	♀	2,271	285 (12.5%)	50 (2.2%)	61 (2.7%)	35 (1.5%)	15 (0.7%)	40 (1.8%)	25 (1.1%)	59 (2.6%)

B

	Wild-type	*Pold1* exo/exo Start	Early	Late
Other phenotypes (single trait)				
hairless			1	
dilution coat color			6	
minor color (brown)	2	1	3	5
short nose				6
microcephalus				1
keratoconus			1	1
colored cornea				1
hypodontia				1
amelogenesis imperfecta				1
ear hypermia				2
ear pigmentation		1		4
ear dysmorphology				1
loss of auricle				1
kyphosis			1	
short limbs & legs			4	2
leg defect				1
syndactyly				2
hyperdactyly			2	3
finger defect	1			1
other finger dysmorphology	2	1	1	8
abdominal distension				3
abnormal penis			1	
cryptorchidism				5
abnormal testis			1	
hermaphroditism	1			
vaginal inflammation				1
priapism (under anesthesia)				1
rectal prolapse			1	2
tail pigmentation			1	1
vocalization behavior		1		
ataxic gait (left hindlimb)				1
Total (single trait)	**6**	**4**	**23**	**55**

	Wild-type	*Pold1* exo/exo Start	Early	Late
Other phenotypes (complex traits)				
circling behavior + kink				1
priapism + hydrocephalus				8
priapism + hydrocephalus + minor color (white)				1
syndactyly + closed eye + other finger dysmorphology + hydrocephalus				1
syndactyly + cataract				1
amelogenesis imperfecta + closed eye				1
amelogenesis imperfecta + hydrocephalus + minor color (white)				1
kyphosis + abdominal distension				1
kyphosis + cataract				1
rectal prolapse + cataract				1
dilution coat color + hydrocephalus			1	
dilution coat color + cataract			1	
finger defect + minor color (brown)				1
other finger dysmorphology + cut tail				1
other finger dysmorphology + minor color (white)				1
hydrocephalus + closed eye				2
hydrocephalus + cataract				1
hydrocephalus + closed eye + cataract				1
hydrocephalus + kink				1
hydrocephalus + minor color (white)				3
kink + cut tail				1
kink + minor color (white)				1
short nose + closed eye				5
short nose + closed eye + cataract			1	
short nose + cut tail + closed eye				1
short nose + minor color (white)				1
short nose + cataract				2
closed eye + cataract	2		1	12
closed eye + cataract + cut tail				1
closed eye + cataract + minor color (white)			1	1
closed eye + minor color (white)		1		10
cataract + cut tail	1			3
cataract + minor color (white)				11
minor color (white) + cut tail				3
Total (complex traits)	**3**	**1**	**5**	**80**

Table S8.

Breeding line−specific phenotypic anomalies. Phenotypic differences among breeding lines were tested for statistical significance by the χ^2-test. As a rough indication, results from each breeding line were compared to the total mutator or control population by two-sided Fisher's exact test. The Fisher's test results are color-coded for easy reference (orange: higher than the total population with $P<0.05$, deep orange: higher with $P<0.01$, green: lower with $P<0.05$).

	n	Mean generation	Abnormal phenotype	Hydrocephalus	Minor color (white)	Cut tail	Tail kink	Closed eye	Cataract	Other
Mutator line										
mutA	917	14.3	93 (10.1%)	23 (2.5%)	38 (4.1%)	10 (1.1%)	4 (0.4%)	3 (0.3%)	2 (0.2%)	13 (1.4%)
mutP	185	18.1	14 (7.6%)	1 (0.5%)	3 (1.6%)	3 (1.6%)	0 (0.0%)	0 (0.0%)	3 (1.6%)	4 (2.2%)
mutF	484	13.1	43 (8.9%)	4 (0.8%)	11 (2.3%)	8 (1.7%)	5 (1.0%)	3 (0.6%)	7 (1.4%)	5 (1.0%)
mutB	906	11.0	103 (11.4%)	8 (0.9%)	14 (1.5%)	13 (1.4%)	12 (1.3%)	16 (1.8%)	10 (1.1%)	30 (3.3%)
mutC	616	13.1	57 (9.3%)	28 (4.5%)	7 (1.1%)	7 (1.1%)	3 (0.5%)	1 (0.2%)	2 (0.3%)	9 (1.5%)
mutD	468	15.2	64 (13.7%)	9 (1.9%)	8 (1.7%)	5 (1.1%)	6 (1.3%)	10 (2.1%)	4 (0.9%)	22 (4.7%)
mutE	1,081	11.3	216 (20.0%)	35 (3.2%)	84 (7.8%)	8 (0.7%)	0 (0.0%)	20 (1.9%)	14 (1.3%)	55 (5.1%)
χ^2-test			***P*<0.0001**	***P*<0.0001**	***P*<0.0001**	*P*>0.05	***P*<0.01**	***P*<0.001**	*P*>0.05	***P*<0.0001**
Control line										
conA	574	11.0	13 (2.3%)	2 (0.3%)	0 (0.0%)	3 (0.5%)	2 (0.3%)	1 (0.2%)	2 (0.3%)	3 (0.5%)
conE	73	15.9	1 (1.4%)	0 (0.0%)	0 (0.0%)	1 (1.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
conD	219	17.3	5 (2.3%)	1 (0.5%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	2 (0.9%)
conB	420	14.3	11 (2.6%)	3 (0.7%)	3 (0.7%)	2 (0.5%)	0 (0.0%)	1 (0.2%)	1 (0.2%)	1 (0.2%)
conF	125	18.5	4 (3.2%)	0 (0.0%)	3 (2.4%)	1 (0.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
conC	238	16.5	11 (4.6%)	2 (0.8%)	1 (0.4%)	2 (0.8%)	0 (0.0%)	0 (0.0%)	3 (1.3%)	3 (1.3%)
χ^2-test			*P*>0.05	*P*>0.05	***P*<0.01**	*P*>0.05	*P*>0.05	*P*>0.05	*P*>0.05	*P*>0.05

Table S9.

Mutator mouse reproductive data, grouped by the number of generations. Survival rate: percentage of pups reaching 8 weeks of age. Offspring/mating: average number of live offspring at 8 weeks per mating. Fertility ratio: offspring/mating relative to the value for mutator generations 0-2. Differences in the parameters associated with reproductive ability between generations 0-2 and later-generation mutator mice were tested for statistical significance by Fisher's exact test (birth rate), Student's *t*-test [litter size (P0), offspring/mating] and Mann-Whitney's *U* test (survival rate). $^{*}P<0.05$, $^{**}P<0.001$, $^{***}P<0.0001$. Litter size (P0), ± s.d.

	Generation (mean)	*n*	Birth rate	Litter size (P0)	Survival rate	Offspring /Mating	Fertility ratio
Wild-type	7^{th}-20^{th} (14.3)	321	0.79	6.6 ±1.8	0.67	3.60	1.36
Mutator	0^{th}-2^{nd} (1.4)	95	0.67	6.3 ±1.6	0.63	2.64	1
	3^{rd}-6^{th} (4.7)	182	$0.51^{(*)}$	4.9 $\pm 2.1^{(***)}$	$0.47^{(*)}$	$1.31^{(***)}$	0.50
	7^{th}-21^{st} (13.2)	1,181	$0.49^{(**)}$	4.9 $\pm 2.1^{(***)}$	$0.37^{(***)}$	$0.96^{(***)}$	0.36

Table S10.

Breeding line−specific reproductive ability. Differences in the parameters associated with reproductive ability among the breeding lines were tested for statistical significance by the χ^2-test (birth rate), one-way ANOVA (litter size, offspring/mating), and Kruskal-Wallis test (survival rate). Survival rate: percentage of pups reaching 8 weeks of age. Offspring/mating: average number of live offspring at 8 weeks per mating.

Line	*n*	Mean generation (range)	Birth rate	Litter size (P0)	Survival rate	Offspring / Mating
Mutator						
mutA	254	15.3 (9-21)	0.56	5.41	0.54	1.70
mutP	39	16.7 (12-20)	0.51	5.50	0.54	1.54
mutF	107	12.8 (8-17)	0.47	4.68	0.39	1.00
mutB	229	12.1 (7-17)	0.35	5.27	0.32	0.63
mutC	74	13.7 (12-17)	0.47	5.23	0.33	0.77
mutD	82	15.5 (11-20)	0.46	4.35	0.37	0.79
mutE	268	11.6 (7-16)	0.56	4.09	0.20	0.50
			***P*<0.0001**	***P*<0.0001**	***P*<0.0001**	***P*<0.0001**
Total	1,053	14.0 (7-21)	0.49	4.88	0.36	0.95
Control						
conA	92	12.6 (6-20)	0.84	6.86	0.61	3.55
conE	17	15.3 (12-18)	0.71	6.50	0.61	3.18
conD	47	15.7 (11-20)	0.70	6.76	0.67	3.30
conB	68	13.8 (6-20)	0.81	6.53	0.73	3.88
conF	34	15.6 (12-20)	0.71	6.54	0.72	3.47
conC	49	14.5 (8-19)	0.84	6.10	0.71	3.80
			P>0.05	*P*>0.05	*P*>0.05	*P*>0.05
Total	307	14.6 (6-20)	0.79	6.59	0.67	3.60

Table S11.

Genomic classification of substitution mutations. Mutation rates are per nucleotide per generation. Substitution mutations were localized to genomic regions including coding sequences (CDS), untranslated regions (UTR), non-coding RNA (ncRNA), introns, and intergenic regions. The ncRNA region was analyzed independently, because it overlaps with many of the other regions. Homozygous variants in control lines were excluded as in Supplemental Table S5.

		Total		CDS		UTR		Intron		Intergenic		ncRNA	
Sample	Homo/ Hetero	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$	Number	Rate $\times 10^{-8}$
mutC	Homo	1,304	8.43	19	7.96	27	14.91	545	8.74	713	8.08	6	18.21
	Hetero	1,944	11.06	32	11.81	29	14.10	796	11.25	1,087	10.85	4	10.70
mutD	Homo	1,472	8.69	27	10.34	22	11.10	631	9.25	792	8.21	2	5.55
	Hetero	1,633	9.23	25	9.17	27	13.04	686	9.63	895	8.88	4	10.62
conA	Hetero	104	0.58	3	1.09	3	1.44	24	0.33	74	0.73	0	0.00
conB	Hetero	100	0.56	1	0.36	1	0.48	32	0.44	66	0.64	0	0.00

Table S12.

Amino-acid change variants found within gene-coding regions in the EWC region, with the following aberrations: Re, reference genomic sequence; Al, alternate sequence; a.a., amino acid substitution; Cons., conservation of the substituted amino acid among species (mouse, rat, and human; conservation among them is indicated by an "O"). Score: the PROVEAN score (Choi et al. 2012), indicating the effect of the amino acid change; orange indicates a deleterious effect (score < -2.50). KO-mouse phenotypes are cited from the Mouse Genome Informatics (MGI) web site. Phenotypes: green, lethal; pink: disease-like; blue: sterile. All of the listed variants, including conA and conB variants, were confirmed to be *de novo* variants by whole genome sequencing or Sanger sequencing.

Chr	Gene	Re	Al	a.a.	Cons.	Score	Reported phenotypes in homozygous KO mice	Chr	Gene	Re	Al	a.a.	Cons.	Score	Reported phenotypes in homozygous KO mice
mutC (Homo)								**mutC (Hetero)**							
chr2	*Olfr1278*	A	G	H89R	-	-3.12	not reported	chr1	*Kcnb2*	A	G	T32A	O	2.17	Neurological abnormalities
chr4	*E130308A19Rik*	A	G	H267R	O	-0.87	not reported	chr1	*Ccdc108*	C	A	D60Y	-	-1.85	not reported
chr4	*Slc31a1*	A	G	T176A	-	-0.74	Embryonic lethal	chr1	*Srgap2*	G	T	A71D	O	-4.54	Fewer than Mendelian ratio, neurological abnormalities
chr4	*Prdm2*	C	T	A946T	-	0.77	Increased incidence of tumors	chr2	*Phf19*	T	C	S470G	O	-2.30	not reported
chr5	*Wdr19*	A	G	R840G	O	-5.13	Embryonic lethal	chr4	*Wdr65*	T	C	D130G	-	-4.03	not reported
chr6	*Fam21*	T	C	F342S	O	-7.35	not reported	chr5	*Akap9*	A	G	E90G	O	-5.85	Male infertily with abnormal germ cell maturation
chr7	*Kif7*	G	T	A1102D	O	-5.94	Neonatal lethal, exencephaly, polydactyly etc.	chr5	*Atxn2*	T	G	H984Q	O	-6.66	Enlarged fat pad, hepatic steatosis, enlarged vesicles
chr8	*Plcg2*	C	A	P75Q	O	-5.18	Abnormalities in blood cells	chr6	*Foxm1*	G	T	G99V	O	-7.95	Embryonic lethal
chr9	*Olfr883*	T	C	C240R	O	-11.57	not reported	chr7	*Stim1*	A	C	E45D	-	-0.64	Perinatal and postnatal lethal (by 2-week-age)
chr10	*Katna1*	G	T	R438L	O	-5.28	not reported	chr7	*Syt9*	G	T	G222V	O	-8.13	50% embryonic lethal
chr10	*Cep57l1*	C	A	M309I	O	-3.79	not reported	chr7	*Ptdss2*	C	A	L316I	O	-1.78	Infertility in about 10% of homozygous males
chr10	*Ranbp2*	A	C	N107H	O	-3.77	Embyonic lethal	chr8	*Chd9*	A	G	K1061R	O	-2.74	not reported
chr12	*Dio2*	G	T	N184K	O	-1.92	Abnormalities in thyroid metabolism	chr8	*Pard3*	T	C	V315A	-	2.28	Embryonic lethal
chr12	*Ccdc85c*	A	G	I407T	O	-3.38	not reported	chr9	*Ephb1*	G	A	R304W	O	-5.52	Abnormalities in optic tract
chr13	*Vmn1r198*	T	C	V291A	O	-1.53	not reported	chr9	*Rpl14*	A	G	R121G	O	-5.80	not reported
chr13	*Rreb1*	C	A	P383Q	O	-3.27	not reported	chr10	*Metap2*	T	C	E309G	O	-6.12	Embryonic lethal
chr17	*Memo1*	G	A	T295M	O	-1.53	not reported	chr13	*Jarid2*	A	T	K475M	-	-2.46	Partial embryonic lethal.
chr18	*Slc39a6*	T	C	H390R	O	-7.28	not reported	chr14	*Grid1*	G	T	E507D	O	-1.28	Abnormalities in hearing functions
								chr14	*Irg1*	G	A	V464I	-	-0.28	not reported
								chr15	*Adcy6*	A	C	L752R	O	-4.88	Abnormalities in homeostasis, behavior etc.
								chr16	*Atp13a4*	T	C	S995G	O	-2.03	not reported
								chr16	*Olfr181*	C	A	A67S	O	-2.75	not reported
mutD (Homo)								**mutD (Hetero)**							
chr1	*Aspm*	A	G	S3012G	O	-3.72	Reduced body weight and fertility in both sexes	chr2	*Itga8*	C	A	E350X	O	stop	Mostly die by the 2nd day after birth, abnormal kidney etc.
chr2	*Olfr1086*	A	C	I219M	O	-2.72	not reported	chr2	*Kif5c*	G	T	A519S	O	-0.35	Reduced brain size and aberrant numbers of neurons
chr2	*Fastkd5*	T	G	N578H	-	-1.66	not reported	chr2	*Anapc1*	A	C	L566R	-	-3.16	not reported
chr3	*Smc4*	C	A	A676D	O	-0.14	not reported	chr3	*Ddah1*	T	G	V131G	O	-6.68	Embryonic lethal
chr3	*Tmod4*	G	T	Q5H	O	-1.55	not reported	chr5	*Ccdc64*	T	G	E365A	O	-3.65	not reported
chr3	*Setdb1*	C	T	G971R	O	-0.87	Embryonic lethal	chr5	*Gal3st4*	G	T	P266T	-	0.07	not reported
chr4	*Focad*	G	T	D1546Y	O	-3.51	not reported	chr6	*Irak2*	T	C	C453R	O	-10.29	Decreased susceptibility to endotoxin shock
chr6	*Usp5*	T	C	M266V	O	-3.07	Embryonic lethal	chr7	*Pth2*	G	C	M1I	O	start	Both sexes are sterile, abnormal germ cell maturation
chr7	*Sytl2*	G	T	M662I	O	-1.76	Abnormalities in gastric cells	chr7	*Apobr*	A	C	L373F	O	-1.50	not reported
chr8	*Tenm3*	T	A	D1116V	O	-6.58	Impaired visual performance	chr9	*Gnai2*	T	G	K296T	O	2.70	Growth retardation, abnormal immune system etc.
chr9	*Nfrkb*	C	T	A752V	O	-1.37	not reported	chr9	*Tcaim*	T	G	L75X	O	stop	not reported
chr9	*Myo9a*	A	C	D2330A	O	-6.01	not reported	chr11	*Atad5*	A	G	K26R	O	-2.76	Embryonic lethal
chr9	*Csnk1g1*	C	A	D2E	O	-1.14	not reported	chr13	*Dmgdh*	T	C	F738L	O	-5.43	not reported
chr10	*Ybey*	T	C	K131R	-	-0.86	not reported	chr13	*S100z*	C	T	D88N	O	-1.67	not reported
chr10	*Actr6*	C	T	G264R	O	-1.62	not reported	chr13	*Mier3*	A	G	H510R	O	-1.99	not reported
chr11	*Ddx42*	A	C	E883D	O	-0.39	not reported	chr14	*Sec24c*	A	C	S978R	O	-3.93	not reported
chr11	*Fasn*	G	T	A1061D	O	-4.25	Embryonic lethal	chr16	*Bach1*	A	G	N257S	-	-0.32	Abnormal expression levels of HMOX1
chr13	*Vps41*	A	G	M673V	O	-0.82	Embryonic lethal	chr19	*Slc22a8*	C	A	L225M	O	-1.92	Decrease in urinary urate levels
chr17	*H2-Eb1*	A	G	Q218R	-	-0.96	not reported	chr19	*Gbf1*	C	A	H645N	O	-1.39	not reported
chr18	*Pcdhgb6*	A	G	Y606C	O	-7.88	not reported								
chr19	*Rbm4*	T	A	S243C	-	-0.70	Reduced body weight, abnormal glucose tolerance								
conA (Homo)								**conA (Hetero)**							
chr1	*Rbm44*	C	G	P873R	-	0.08	Enhanced fertility with increased litter size	chr13	*Gpr98*	G	A	R801W	O	-7.90	Abnormal cochlear hair cells
chr1	*Crb1*	C	T	C912Y	O	-9.50	Abnormalities in retinal functions	chr18	*Tcof1*	C	T	G924R	-	-0.33	Heterozygous null mutant die perinatally with craniofacial
chr12	*Papln*	T	G	W367G	O	-12.76	not reported								
conB (Homo)								**conB (Hetero)**							
chr1	*Rbm44*	C	G	P873R	-	0.08	Enhanced fertility with increased litter size								
chr1	*Crb1*	C	T	C912Y	O	-9.50	Abnormalities in retinal functions								
chr2	*Nusap1*	C	T	T85M	O	-3.80	Embryonic lethal								
chr7	*Mogat2*	G	A	R101W	O	-4.39	Resistance to diet induced obesity								
chr11	*Ern1*	C	T	V392M	O	-1.74	Embryonic lethal								
chr12	*Pnn*	C	T	R91W	O	-5.21	Embryonic lethal								

Table S13.

Identified *de novo* structural variants (deletions) in the breeding lines.

Sample	Homo/Hetero	Chr.	Start position	Type of variant
mutD	Homo	chr5	123,470,996	204-bp deletion
mutD	Homo	chr5	123,514,089	1,013-bp deletion

Table S14.

Estimated number of initial variants in the mutC and mutD lines. Details are shown in Supplemental Information, "Effect of initial variants on mutator-line phenotypes." In this case, the variants were called independently in each individual and in "Adam/Eve," so there are minor differences from the number of candidate *de novo* variants shown in Supplemental Table S3.

		SNVs			Indels		
		Total	B6N	Other than B6N	Total	B6N	Other than B6N
Line mutC	*De novo*	1,306	0	1,306	28	0	28
	Initial	806	414	392	29	16	13
Line mutD	*De novo*	1,477	0	1,477	21	0	21
	Initial	872	457	415	36	18	18
Common in mutC & mutD	*De novo*	67	0	67	1	0	1
	Initial	583	294	289	25	14	11

Table S15.

Summary of the regression analysis. Details are shown in the Supplemental Methods, "Regression analysis."

Phenotypes	Model		Mutator [95%CI]	Control [95%CI]
Visible abnormal (%)	Binomial linear	*n*	6,229	1,649
		β_0	4.36 [3.03, 5.60]	0.79 [+0.00, 2.60]
		β_1	0.68 [0.55, 0.81]	0.14 [0.00, 0.27]
		(*P* value)	$<1.1\times10^{-16}$	0.0628
Weight (g)	Linear	*n*	1,308	611
－male		β_0	22.01 [21.66, 22.36]	23.16 [22.46, 23.86]
		β_1	-0.115 [-0.144, -0.086]	-0.032 [-0.075, 0.010]
		(*P* value)	1.05×10^{-14}	0.137
－female		*n*	1,393	549
		β_0	17.99 [17.74, 18.23]	18.71 [18.17, 19.24]
		β_1	-0.090 [-0.110, -0.070]	-0.028 [-0.061,0.004]
		(*P* value)	4.09×10^{-18}	0.0916
Reproduction (number of offspring)	Negative binomial linear	*n*	1,458	321
		β_0	1.58 [1.19, 1.97]	3.46 [1.91, 5.00]
		β_1	-0.042 [-0.071, -0.014]	0.010 [-0.094, 0.115]
		(*P* value)	4.30×10^{-4}	0.837
		AIC	3,803.3	-
	Recessive lethal mutation model	*n*	1,458	-
		β_0	2.83 [1.61, 4.05]	-
		U	1.98 [1.14, 2.81]	-
		(*P* value)	5.13×10^{-7}	-
		AIC	3,790.3	-

Table S16.

The overdispersion value was determined in several settings to estimate confidence intervals (CIs) for the mutation rate (μ). r: recombination rates (cM/Mb). Details are shown in the Supplemental Methods, "Confidence Intervals for μ and combined estimates."

Corresponds to:	μ ($\times 10^{-8}$)		r=0.5	r=0.6	r=0.7	r=∞	CI calculation
SNVs in mutant lines	10	hetero	4.11	3.82	3.60	1.00	simulation
		homo	2.02	1.96	1.83	0.99	simulation
SNVs in control lines	0.5	hetero	1.31	1.27	1.21	1.02	simulation
		homo	1.08	1.05	1.03	0.97	Poisson
Indels in mutant lines	0.25	hetero	1.13	1.10	1.10	1.01	Poisson
		homo	1.07	1.02	1.02	1.00	Poisson
Indels in control lines	0.05	hetero	1.01	1.01	0.99	0.99	Poisson
		homo	0.99	1.04	0.98	0.97	Poisson

Legend for Movie S1

Human audible vocalization exhibited by a mutant. The vocalization behavior of a ten-week-old male mouse is presented. The vocalization begins in the mutants after sexual maturation (at about 8-weeks of age) in both sexes.

Legend for Supplemental Material

Information for the Sanger sequencing of the SNVs and indels.